

UNIVERSITE DE GENEVE
Département de médecine
Division d'immunologie et d'allergie

FACULTE DE MEDECINE
Professeur A.F. MULLER
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**IMMUNE-COMPLEX GLOMERULONEPHRITIS
ASSOCIATED WITH
HEPATITIS B VIRUS INFECTION.
REPORT OF A CASE AND
REVIEW OF THE LITERATURE**

THESE

présentée à la Faculté de médecine de l'Université de Genève
pour obtenir le grade de docteur en médecine

par

Léon-Nick BENUSIGLIO

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Thèse No 3531

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La Faculté de médecine, sur le préavis du professeur Alex F. Müller et du professeur André Cruchaud, autorise l'impression de la présente thèse, sans prétendre par là émettre d'opinion sur les propositions qui y sont énoncées.

Genève, le 21 septembre 1976

Le doyen :
Marcel Jenny

Thèse No 3531



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A MA FEMME, SONIA

A MON FILS, SERGE

A MES PARENTS

Introduction.

Some viral infections are known to cause glomerulonephritis (GN) in animals. This is the case with Aleutian mink disease¹, infectious equine anemia², chronic hog cholera³, as well as murine lymphocytic choriomeningitis⁴, lactic dehydrogenase virus^{4,5} and Echovirus 9 infections⁶. The occurrence of GN in viral diseases has also been described in man⁷⁻¹².

Combes et al.¹³ demonstrated the association of virus antigen with immune deposits in the kidney. They reported the case of a 53-year old patient who developed hepatitis four months after blood transfusions and who had persistent hepatitis B surface antigen (HB_sAg) in his serum. Severe proteinuria occurred 22 months later. A renal biopsy revealed membranous GN, and immunofluorescence disclosed HB_s Ag in the glomeruli in a pattern similar to C3 and immunoglobulin G (IgG). Antibodies to HB_s Ag (anti-HB_s) and immune complexes could not be found in the serum; complement levels were normal.

Liver biopsy showed periportal fibrosis and bile duct proliferation. Since then, at least seven cases of adults and children suffering from chronic hepatitis with kidney involvement have been reported¹⁴⁻¹⁶. All had deposits of HB_s Ag, IgG, C3 and/or IgA and IgM on their glomerular basement membranes (GBM). Brzosko et al.¹⁷ studied 52 children with GN but without any apparent liver disease; HB_s Ag was found in the glomeruli of 18. All 18 had antibodies to the hepatitis B core antigen (HB_c Ag) in their serum, and 16

were HB_s Ag positive. Relevant data from the literature are presented in tables 1 and 2.

In several of the previous reports detailed morphological investigations contrasted with rather incomplete serological data. This prompted us to correlate immunofluorescent findings with complement studies, and to compare our data with those of other authors.

Case report.

A 45-year old Spanish man was hospitalized in November 1975 with a diagnosis of nephrotic syndrome.

He emigrated from Spain to Switzerland in 1958. He was well until 1968, when he contracted hepatitis. At this time, peak serum bilirubin was 5 mg/100 ml, peak serum glutamic oxalacetic transaminase (SGOT) was 490, and peak pyruvic transaminase (SGPT) was 650 IU/l. Tests for HB_s Ag were not done. Ten weeks later, SGOT and SGPT were still high (60 and 80 IU/l).

The patient resumed full time work as a porter in a hotel and felt healthy. In the fall of 1975 he noticed increasing fatigue, anorexia, and a weight gain of 6 kg, and his physician found proteinuria, hypoproteinemia and hyperlipidemia. He was then hospitalized on October 18th, 1975. Physical examination revealed a patient in fair general condition weighing 57 kg for a height of 155 cm. Important pitting edema was present on ankles and tibias. Blood pressure was

200/100 mm Hg. Salient laboratory data were as follows:

Serum albumin 1.9 and serum globulin 2.3 g/100 ml, cholesterol 340 mg/100 ml, and creatinine clearance 70 ml/min.

A test for HB_s Ag was positive by radioimmunoassay. Tests for antinuclear antibody, rheumatoid factor, LE cells, syphilis, cryoglobulins, antistreptolysin O, smooth muscle antibodies and mitochondrial antibodies were all negative.

Proteinuria ranged from 4 to 6 grams per day. Innumerable red cells, 10 white cells and some red cell casts and birefringent casts were found in the urinary sediment.

Urinary lysozyme was not found. A needle biopsy of the liver showed slight fibrosis with little inflammation. A renal biopsy was also performed.

The patient was treated with bed rest and low salt-high protein diet. Edema disappeared, blood pressure dropped to 140/90 mm Hg, and the patient lost 7 kg. A follow-up examination two months later revealed that he had not regained any weight on a low salt diet with 50 mg of chlorthalidon 3 times per week. The plasma protein level was 5.1 g/100 ml, HB_s Ag was still positive, proteinuria was 2 g/24h, and SGOT and SGPT were 32 and 31 IU/l respectively. Six months later there was no important clinical or laboratory change. A second renal biopsy was performed; only immunofluorescent pattern was examined.

Materials and methods.

Tissue samples obtained by needle biopsy were fixed in Dubosq-Br  zil solution for histology and quick-frozen in OCT compound (Ames Company, Elkhart, Indiana) for immunofluorescence.

Immunofluorescence: Sections, 5 microns thick, were prepared in a cryostat. They were incubated with antisera conjugated with fluorochromes for 30 minutes in a humid atmosphere. The excess of conjugates was then removed by three washings in phosphate buffered saline (pH 7.4). Sections were counterstained by immersion in Evan's blue for five minutes. Preparations were mounted in buffered glycerol and examined with a Zeiss photomicroscope equipped with an epi-illuminator and interference filters for fluorescein and rhodamine.

Antisera: Monospecific antisera against IgG, IgA, IgM, kappa light chains and C3 were obtained and conjugated to fluorochromes as already described^{18,19}. Fluorescent rabbit antifibrinogen and rabbit anti-HB_s antisera as well as human serum containing HB_sAg were obtained from Boehringerwerke, Marburg-Lahn, Germany. Fluorescein-conjugated swine anti-rabbit immunoglobulin (Swarig-F) and rabbit anti-monkey Ig (Ramig-F) were obtained from Nordic Pharmaceuticals and Diagnostics, Tilburg, The Netherlands. Monkey anti-HB_c antiserum was a generous gift of Dr. J. Gerety, National Institute of Allergy and Infectious Disease, Bethesda, MD. Anti-rabbit

and anti-monkey sera were absorbed with human Ig before use.

Complement: CH 50 was measured by a hemolytic technique²⁰ (normal ± 2 standard deviations = 44 ± 8 U/ml). C3 was determined using the B_{1A}-Partigen plates (Boehringerwerke, normal = 85 ± 22 mg/100 ml). C4 was determined by radial immunodiffusion using commercial anti-C4 serum (Boehringerwerke) and expressed as a percentage of the C4 level of a pool of normal sera (normal = $100 \pm 52\%$)^{21,22}. C3 pro-activator (C3PA) was determined according to Perrin²¹ and expressed as a percentage of normal (normal = $100 \pm 36\%$). C3d was determined according to Perrin²³ (normal = less than 1,2 mg/100 ml). Circulating immune complexes were measured by a modified Clq binding test²⁴.

Results.

Light microscopy (Fig. 1): 23 glomeruli could be studied on the first biopsy. Two were completely hyalinized. The main lesions were: (1) a moderate increase of mesangial matrix together with mesangial cell proliferation and (2) a segmental thickening of capillary walls with subendothelial deposits and splitting of the GBM. In about 20 percent of the glomeruli, these lesions were accompanied by epithelial crescents. In addition there was a mild degree of arteriosclerosis. These findings are compatible with the diagnosis of membranoproliferative GN with epithelial crescents.

Immunofluorescence: Direct immunofluorescence with anti-IgG, -IgM, -C3 and -fibrinogen disclosed abundant granular deposits along the GBM in all glomeruli (Fig.2). A few such deposits were also visible in the mesangium. Small quantities of IgM and C3 were seen around a few tubules and vessels, and some IgA was detectable at the periphery of some glomeruli.

We looked for HB_s Ag by indirect fluorescence. Sections were incubated first with unconjugated rabbit anti - HB_s and then with Swarig-F. A strong granular fluorescence was observed (Fig. 3). To ascertain that HB_s Ag and Ig had the same localization, sections were first incubated with rabbit anti-HB_s; the latter was identified by Swarig-F. Then, the sections were stained for Ig with Rahig-R. Observation of glomeruli at high magnification (x800) using the double-filter system of the Zeiss photomicroscope revealed that rhodaminescent human Ig and fluorescent granules of HB_sAg had the same location. Controls consisted of incubating kidney sections with (1) normal rabbit serum and Swarig-F, (2) Swarig-F only, and (3) rabbit anti-HB_s absorbed with human serum containing HB_s Ag, followed by Swarig-F. All these controls gave negative results.

HB_c Ag was looked for on a second kidney biopsy performed six months after the first one. Sections were covered with monkey anti-HB_c and then with Ramig-F. No HB_c Ag could be detected by this method. However, on this biopsy, staining

for HB_s Ag was also negative although HB_s Ag was still present in serum and urine.

Serological data (Table 3): Hemolytic complement was decreased at the time of hospitalisation as well as two and six months later. C3 was decreased and the Clq binding test was positive in October and December 1975. These tests were practically normal in April 1976. C4 was decreased in 1975 and in April 1976 while C3 proactivator was constantly increased. This suggests that complement was activated only through the classical pathway.

Discussion.

In our patient GN occurred seven years after acute hepatitis. In previous cases (see table 1) the interval between acute hepatitis and GN ranged from 13 months to 20 years. Often, no episode of acute hepatitis could be identified, and signs of chronic hepatitis were discovered at the same time as GN^{14,25}. Our patient was clinically free of liver disease, with only minimal fibrosis on liver biopsy. He is at one end of a spectrum ranging from the simple HB_s Ag carrier state¹⁶ to severe chronic active hepatitis²⁵.

In the other cases, complement levels have been low or normal. Circulating complexes were found by Bläker (see table 1). Our patient's serum contained immune complexes, and complement was constantly low; in his glomeruli HB_s Ag was in the same

position as Ig₁ and C3, as judged by immunofluorescent patterns. It is therefore tempting to conclude that HB_s Ag - antibody complexes were deposited in the kidney like DNA - antiDNA complexes in lupus GN²⁶. However, while antiDNA antibodies are readily found in the serum of most patients with lupus GN²⁷, anti-HB_s antibodies are usually absent from the sera of patients with HB_sAg²⁸; patients with HB_s Ag and GN conform to this rule (see table 1). Two explanations may reasonably account for this difference:

1. All anti-HB_s antibodies are fixed in the kidney. An analogous situation exists (1) in certain acute rejections of kidney transplants where anti-kidney antibodies appear in the serum only after removal of the kidney²⁹, (2) in the GN of acute serum sickness³⁰, and (3) in some cases of systemic lupus erythematosus, where anti-DNA titers fall during exacerbation of GN³¹.
2. The GN associated with hepatitis B may not be due to HB_s Ag-antibody complexes. At least four HB-associated antigens have been identified: HB_s, HB_c, e-antigen, DNA polymerase^{32,33}. HB_s Ag might just be a marker for another HB-associated antigen that is responsible for the GN.

The follow-up of our patient tended to support the second hypothesis. At the time of the second renal biopsy, serum protein level and proteinuria had not changed. On kidney sections, HB_s Ag could not be detected but staining for Ig and C remained positive. Thus, clinical evidence of chronic GN persisted although HB_s Ag was no longer found in the

kidney. This suggests that another antigen was maintaining the GN. Staining for Ig and C was less intense than on the first biopsy: it seems unlikely that all HB_s Ag became covered by Ig and was therefore inaccessible to rabbit anti-HB_s.

Chronic GN is not the only immunologic disorder associated with HB virus infection. Arthralgias are common in patients with acute viral hepatitis³⁴. Serum complement may be low^{34,35}, and a segmental GN with hemorrhages and deposits of Ig and C has been described³⁶. This serum sickness-like syndrome disappears spontaneously in three to seven days.

30 percent of patients with polyarteritis nodosa are chronic carriers of HB_s Ag³⁷⁻³⁹. Evidence of HB infections may precede or follow the signs of vasculitis. Some of these patients suffer from GN with massive proteinuria, and have granular deposits of Ig and C in their glomeruli. However, the search for circulating HB_s Ag-antibody complexes has not been successful³⁷. Prognosis is highly variable; some patients survive for many years without evidence of either hepatitis or recurrent vasculitis, while others die of liver or vascular disease.

Some cryoglobulins contain HB_s Ag^{40,41}. Cryoglobulinemia may be associated with HB_s antigenemia; acute GN may develop after exposure to cold.

In our case, light microscopy led to a diagnosis of membrano-proliferative GN. Many of the previously published cases (see table 2) were membranous GN; however, the correlation between etiology and histology of GN is weak²⁶.

The prognosis of GN associated with HB is unknown. At least one patient has progressed to renal insufficiency and transplantation²⁵; some have needed immunosuppressive therapy. Our patient was treated with thiazides and diet only. Six months after his first biopsy, renal function and proteinuria were essentially unchanged. Thus, he has done rather better than most patients with membranoproliferative GN⁴².

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Abbreviations:

C:	Complement
GBM:	Glomerular basement membrane
GN:	Glomerulonephritis
Ig:	Immunoglobulin
HB:	Hepatitis B
HB _C Ag:	Hepatitis B core antigen
HB _S Ag:	Hepatitis B surface antigen (= Australia antigen)
Anti-HB _C :	Antibody to hepatitis B core antigen
Anti-HB _S :	Antibody to hepatitis B surface antigen
Rahig-R:	Rhodamine-conjugated rabbit anti-human kappa chain serum
Ramig-F:	Fluorescein-conjugated rabbit anti-monkey immunoglobulin serum
Swarig-F:	Fluorescein-conjugated swine anti-rabbit immunoglobulin serum

Table 1: Clinical and serological data.

Author (Ref)	Number of cases	Age	Sex	Serum creat mg%	SGOT U/l	Interval HB-GN 1) months ¹⁾	C	Immune complexes (serum)	HB _s Ag Ab	Treatment
Combes (13)	1	53	M	1.3	200	11	normal	-	+	- Prednisone
Myers (14)	1	30	F	2.5	15	unknown	low	ND	+	ND
Knieser (25)	3	24	M	0.8	125	unknown	ND	ND	+	- Prednisone Cyclophosphamide
Brzosko (17)	18	18	F	25	177	24	ND	ND	-	+ Renal transplant
		22	M	3.6	268	unknown	ND	ND	+	- Azathioprine
		children		ND	ND	ND	ND	ND	+ ³⁾	ND ND
Kohler (15)	1	42	M	1.1	ND	240	normal	-	+	- none
Bläker (16)	2	7	ND	0.9	30	unknown	normal	+	+	ND ND
		15	ND	0.5	42	unknown	normal	+	+	ND ND
Present case	1	45	M	0.9	20	84	low	+	+	- Low-salt diet diuretics

+ = positive or present

- = negative or absent

ND: not done or not mentioned

1) Interval between onset of acute viral hepatitis and detection of GN.

2) HB_s antigen and antibody in serum3) 16 of 18 with glomerular deposits of HB_s Ag (see text).

Table 2 : Liver and kidney biopsies : immunofluorescent and histological findings.

Author (Ref)	Kidney : histological diagnosis	Kidney : immunofluorescent deposits					Liver histology
		IgG	IgA	IgM	C3	Fibrin - HB _s Ag ogen	
Combes (13)	membranous GN	+	-	-	+	-	mild fibrosis and inflammation, no necrosis
Myers (14)	membranoproliferative GN	+	ND	ND	+	ND	cirrhosis intense inflammation necrosis
Knieser (25)	membranous GN	+	+	+	+	ND	chronic active hepatitis
	interstitial nephritis	-	+	+	+	ND	persistent hepatitis
	membranoproliferative GN	+	+	+	+	ND	chronic active hepatitis
Brzsko (17)	12 membranoproliferative GN	+	+	+	+	ND	ND
	2 membranous GN						
	4 proliferative GN (2 extra- and 2 endocapillary)						
Kohler (15)	membranous GN	+	ND	+	+	ND	portal inflammation
Bläker (16)	membranous GN	+	•	-	+	+	ND
Present case	membranoproliferative GN	+	(+)	+	+	+	mild fibrosis minimal inflammation

Symbols as in Table 1.

Table 3 : Serum complement levels and circulating immune complexes

Date	10/1975	12/1975	4/1976
Hemolytic complement ($\bar{N} \pm 2 \text{ SD} = 44 \pm 8 \text{ U/ml}$)	32	33	32
C3 ($\bar{N} \pm 2 \text{ SD} = 85 \pm 32 \text{ mg/100ml}$)	53	52	78
C3 Proactivator ($\bar{N} \pm 2 \text{ SD} = 100 \pm 36\%$)	135	140	105
C3d ($\bar{N} \pm 2 \text{ SD} = 0.8 \pm 0.5 \text{ mg/100ml}$)	1.4	ND	0.4
C4 ($\bar{N} \pm 2 \text{ SD} = 100 \pm 52\%$)	33	37	22
Clq-binding test ($\bar{N} < 11\%$)	24	18	14

Fig. 1 (left) - Two glomeruli with increase of mesangial matrix and irregular thickening of the basal membrane (PAS stain x 220).

(right) - Detail of a glomerulus showing capillary walls with double contours (arrow). There are no argyrophilic deposits (Silver stain x 580).

Fig. 2 Immunofluorescent staining of glomeruli for C3 (top left, x 220), IgG (top right, x 800), IgM (bottom left, x 800), and HB_s Ag (bottom right, x 800).

Summary.

Some cases of glomerulonephritis (GN) have been ascribed to the deposition of hepatitis B surface antigen (HB_s Ag) - antibody complexes on the glomerular basement membrane (GBM). We present the case of a 45-year old man with membranoproliferative GN seven years after acute hepatitis. He was found to be a chronic carrier of HB_s Ag. Glomeruli contained granular deposits of immunoglobulin (Ig), complement (C) and HB_s Ag. Six months later, GN persisted. HB_s Ag had disappeared from glomeruli, while Ig and C were still present. We conclude that this GN is probably due to a hepatitis B associated antigen, but not necessarily to HB_s Ag.

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